

UNIVERSITY OF PENNSYLVANIA

A STUDY OF THE LABORATORY
AIDS TO THE DIAGNOSIS OF
CHRONIC GONORRHEA
IN WOMEN

RAYMOND SEARS PATTERSON

A THESIS

IN BACTERIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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In acute gonococcus infections a clinical diagnosis usually may be confirmed by a microscopical examination of a stained smear, but in chronic gonorrhea no single laboratory test can be relied upon to confirm the clinical diagnosis. Torrey, Wilson, and Buckell (1) studied the available laboratory procedures and conclude that whenever possible the smear, cultural, and complement-fixation tests should all be carried out as they tend to supplement each other; and that the value of the several tests increases in the order named, the complement-fixation test being most useful.

The conclusion of these investigators confirms the premise of this paper that there is no single, satisfactory test available for the confirmation of the diagnosis of chronic gonorrhea in women. As the control of the spread of gonorrhea is dependent upon a satisfactory test to detect gonorrhea in persons presumed to be infecting others, the need prompted a reconsideration of the value of the accepted laboratory procedures and a study of an additional diagnostic method which hitherto has not been reported as applied to the diagnosis of gonococcus infections.

In common with several States, the New Jersey law² requires physicians to report to the State Department of Health cases of gonorrhea (and other venereal diseases) and provides that the source of infection, if known, shall be reported as well. The

¹ Presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment for the degree of Doctor of Philosophy.

² Chapter 232 of the Laws of New Jersey of 1917.

New Jersey State health department, emphasizing the importance of this provision of the law, urges physicians to try to ascertain the source of infection of all cases of gonorrhea and recently acquired syphilis, and each year sources of venereal infection are recorded in increasing numbers.

As gonorrhea in women frequently remains undetected and as sexually promiscuous women usually do not know who infected them, the returns for the most part are confined to the names of prostitutes—both clandestine and professional—given by the men whom they have infected. A detailed consideration of these facts is set forth in an earlier paper (2).

The investigation of reported clandestine prostitutes by the local health officials having jurisdiction over them, or the apprehension of professional prostitutes by the appropriate police authorities, leads to a medical examination of the suspected persons. In practice, the health board physician or the police surgeon makes a superficial examination, and prepares a smear of the cervical or urethral exudate which is sent to a diagnostic laboratory. A negative report upon the examination of the smear satisfies the official that the suspected person is not suffering from gonorrhea, and this terminates the control of a person presumably capable of infecting others.

The first consideration in this study of the accepted laboratory procedures—the smear, the culture, and the blood complement-fixation test—was the practicability of the test as applied upon a State-wide scale to be made available where, and as, the test is needed. Thus, in assuming that the microscopical examination of the smear stained by Gram's method is unsatisfactory because positive findings are so infrequently obtained, the possibilities of better results by careful technique in obtaining specimens from women patients are not forgotten. If smears of the cervical discharge are taken the day following the cessation of the menstrual flow, the patient having left the parts unwashed and avoided local treatment, and if the smears are made of freshly expressed pus evenly and thinly spread, positive findings will be obtained in a larger proportion of cases than if these precautions are not observed.

MICROSCOPICAL METHODS FAIL TO DETECT MOST CHRONIC
GONOCOCCUS INFECTIONS IN WOMEN

Only in a small proportion of all clinically positive chronic gonococcus infections can the diagnoses be confirmed by microscopical examination of stained smears. Thompson (3) reviews the literature on the subject and concludes that:

It is extremely difficult to diagnose gonorrhea in the female by smear examinations, whether the sample of pus be taken from the urethra, vagina, or cervix. The majority of such smears when examined by the microscope show myriads of organisms, diplococci and diplobacilli (Gram-positive and Gram-negative), and only a bold and inexperienced observer would dare to diagnose the presence of isolated gonococci in such a mass of bacteria. The diagnosis is only certain where numbers of gonococci are found intracellularly. These may be found in the early acute stage, but it is rare to find such a picture in chronic female cases. In the majority of chronic female discharges the author has found the smears negative to gonorrhea or doubtful.

This statement is so generally accepted that it hardly needs confirmation here, however it may be worth while to note the results of the microscopical examination of smears of a series of cases used in another part of this study: Positive or doubtful findings were recorded in less than one-fifth of the specimens, which were of freshly expressed pus from the cervix of clinically positive, chronic gonorrheal cases in women.

CULTURAL METHODS PRESENT MANY PRACTICAL DIFFICULTIES

Cultural methods properly carried out by an experienced bacteriologist under the most advantageous conditions frequently result in positive findings in cases missed by the microscopical examination of smears, but the practical difficulties encountered in this method tend to minimize its value as a practical aid to the diagnosis of chronic gonococcus infections. The conditions requisite to success are: freshly prepared, suitable culture media; close connection between clinic and laboratory; careful technique in obtaining specimens; skilful manipulation of cultures; and

ample time for incubation of original and sub-cultures. Even under these conditions the proportion of positive cultures obtained from chronic gonococcus infections is small.

Torrey, Wilson, and Buckell report 50 per cent positive findings in subacute cases and 12 per cent positives in chronic cases. Thompson reviews the reports of earlier observers, and in none is the proportion of positive findings sufficiently large to indicate the culture method as the one of choice as an aid to diagnosis. As a method of diagnosis offered on a State-wide basis, the difficulties of distributing fresh culture media, transportation to laboratories, and the time necessary to secure a finding practically prohibit its use. In the series of cases available for this study, the examinations were made at widely separated places and at irregular hours, therefore it was found impossible to obtain specimens from which cultures could be grown successfully.

THE COMPLEMENT-FIXATION TEST OF BLOOD IS OF GREATEST DIAGNOSTIC VALUE

The complement-fixation test of the blood of suspected cases of chronic gonorrhea gives a much higher proportion of positive findings than either the microscopical or cultural methods. The results of the application of the test in this study confirm the findings of Teague and Torrey (4), Schwartz and McNeil (5), Smith and Wilson (6), and Wilson, Forbes and Schwartz (7).

The method used here is a modification of that of McNeil as described in Park and Williams (8) and was selected after a comparative trial with other methods and antigens because in our hands it gave the most concordant results.

Brief description of reagents and the test³

Antigen. Three strains of gonococci isolated by Torrey⁴ are grown on ascitic-agar slants and transferred after forty-eight hours to a num-

³ Detailed descriptions of this method of conducting the blood complement-fixation reaction may be found in Park and Williams—see reference (8).

⁴ Dr. J. C. Torrey kindly supplied cultures of the three strains found by him to be most highly generalized from the antigenic standpoint.

ber of plates of the same medium. After forty-eight hours incubation the growth is removed, extracted in absolute alcohol and ether, dried, and ground. The powder is suspended in saline in the proportion of 1 gram of powder to 200 cc. of saline and heated at 80°C. for one hour.

Immune serum. To provide immune serum for the positive control a rabbit was given a series of three intravenous injections of commercial gonococcus vaccine at five-day intervals, the dosage being 0.5 cc., 1.0 cc., and 1.0 cc. of a vaccine containing 1000 million gonococci per cubic centimeter. One week after the last injection, the serum being found satisfactory, the rabbit was bled from the heart under an anesthetic. The blood was allowed to clot and the immune serum was poured off, centrifugalized, and inactivated at 56°C. for one-half hour, on three successive days.

Complement. The blood from several guinea pigs is needed and each should be kept separate. After clotting, the serum from each is poured off and centrifugalized.

Hemolytic system. Sheep erythrocytes in five per cent suspension and hemolytic amboceptor obtained from rabbits immunized against sheep cells are used.

*Titration of reagents.*⁵ The titration of the hemolytic amboceptor is similar to that practiced in the routine Wassermann reaction. Varying dilutions of the hemolytic serum are added in amounts from 0.09 to 0.01 cc. to 0.1 cc. each of cells and a 1:10 dilution of complement. That dilution is used in which the tube containing 0.05 cc. causes complete hemolysis, but in which the 0.04 cc. tube shows slight cloudiness. At this dilution two hemolytic units are contained in 0.1 cc., the amount used in the test.

Preliminary titrations of complement. Each serum is tested individually to insure the absence of natural antish sheep amboceptor, hemolytic activity, and the ability to fix, or combine, with gonococcus antigen and immune serum. Sera which contain no interfering amount of antish sheep amboceptor cause no hemolysis when 0.2 cc. of a 1:10 dilution is added to 0.1 cc. of cell suspension; and those which cause rapid hemolysis when added in 0.1 cc. amounts to 0.2 cc. of sensitized cells are actively hemolytic. Satisfactory sera are then titrated for anti-complementary properties and fixability by the method on page 298.

⁵ To conserve space, the fixation periods and temperatures are omitted: in all titrations the fixation period is one hour at a temperature of 37°; after the sensitized cells are added the test is returned to the water bath for half an hour.

Preliminary test of complement

TUBE	ANTIGEN DILUTION	IMMUNE SERUM (1:10 DILU- TION)	COMPLE- MENT (1:10 DILU- TION)	SALINE	SENSITIZED CELLS	
	cc.	cc.	cc.			cc.
1	0.4		0.1	To make up 0.4 cc.	One hour at 37°C.	0.2
2	0.3					
3	0.2					
4	0.1					
5		0.04				
6	0.2	0.02				
7	0.1	0.02				
8	0.05	0.02				
9	0.025	0.02				

In this test the complement must show very little, if any, fixation with 0.4 cc. of antigen (tube 1), none with 0.04 of serum (tube 5), and at least partial fixation with 0.025 cc. antigen plus 0.02 cc. of serum (tube 9). Almost half the guinea-pig sera tested were either too anti-complementary or did not fix readily and were discarded. Those complements which are satisfactory are pooled, diluted with saline, 1:10, and then titrated. The complement is titrated by adding 0.2 cc. of sensitized cells to varying quantities (0.09 to 0.01 cc.) of a 1:10 dilution of the pooled sera. The tube containing the least amount of complement in which hemolysis is complete is selected as the basis for further dilution, but the next tube containing the next smaller is also used as a basis for the second or higher dilution, if (as it should be) this tube is almost completely hemolyzed. The complement is made in two dilutions on the basis of this titration.⁶

After titrating and diluting the complement, the titration is repeated with the two dilutions as a check on the first titration. If the check is satisfactory, the test is carried on.

Having determined the units of hemolytic amboceptor and complement, it is possible to determine the proper dilution of antigen and im-

⁶ If the tube containing 0.05 of the complement is the unit, the 1:10 dilution of complement is satisfactory; if the 0.04 tube is the unit, the complement is diluted 1:12½; if the 0.03 tube is the unit, the dilution is made 1:16½. Complement stronger than this is rare and probably unsatisfactory. As the least point at which hemolysis occurs is not definitely at 0.04 or 0.03, it probably occurs between the two; therefore, two dilutions are used, the one based upon the titration, and the other the next higher dilution. In our experience this combination gave the most satisfactory results, for in the lower dilution the excess of complement frequently is too great to be fixed by some sera; whereas, others are anticomplementary in the higher dilution but give a readable reaction in the lower.

immune serum. The stock antigen may be diluted further, and in this test two different lots were found to give good results when diluted 1:20 with saline. The titre of the dilution can be determined by combining, with complement and sensitized cells, from one to four times the unit (0.1 cc.) of antigen dilution without immune serum, and from two units to one-quarter unit in combination with 0.01 cc. of immune serum. If the antigen shows only a trace of fixation in four times the unit without immune serum, and complete fixation in the tubes containing less than 0.1 cc. of antigen plus serum, then the antigen and the dilution are satisfactory.

The immune serum is titrated by adding 0.01 and 0.02 cc. quantities of varying dilutions of serum to antigen, 0.1 cc., complement, 0.1 cc., and sensitized cells, 0.2 cc. The serum from the rabbit immunized for this test fixed complement when diluted one part serum to nine parts of saline.

The test as we conducted it requires for each serum—including the known negative, known positive, and the unknown sera—six tubes arranged as follows:

*Blood complement-fixation test**

TUBE NUMBER	ANTIGEN	SERUM	COMPLEMENT IN 2 DILUTIONS	SALINE	HEMOLYTIC SYSTEM
	cc.	cc.	cc.		cc.
1		0.04	(Low dilution)	0.16	0.2
2	0.1	0.02	0.1	0.08	
3	0.1	0.01		0.09	
4		0.04	(High dilution)	0.16	0.2
5	0.1	0.02	0.1	0.08	
6	0.1	0.01		0.09	

Controls

1	0.4		(Low dilution)		0.2
2	0.2		0.1		
3	0.1			0.1	
4	0.4		(High dilution)		0.2
5	0.2		0.1		
6	0.1			0.1	
7			0.1	0.2	
8				0.3	0.2
9				0.3	0.1 (cells)

* Fixation period: one hour at 37°C. After adding the hemolytic system the test is replaced in the water bath until the controls are completely hemolyzed.

In reading the results of the reaction the controls are first observed. Control tubes 1 and 4 may show slight degrees of fixation; tubes 8 and 9 should show no hemolysis; all other tubes should be hemolyzed.

The known positive (immune rabbit serum) and known negative serum tests are observed; if these are satisfactory, the tests of the unknown serum are read.

First the anticomplementary serum controls are observed; if tube 4 is completely hemolyzed, the test in which the higher dilution of complement is used may be read. If in that dilution of complement the serum gives evidence of anticomplementary action, but tube 1 is clear, then the tubes with the lower dilution of complement should be read. An appreciable degree of fixation in the quarter amount of serum (tubes 3 or 6) and not less than half the cells remaining unhemolyzed in the half amount of serum (tubes 2 or 5) was read as positive; less than this degree of fixation, but any evident fixation was read as doubtful; when hemolysis was complete in all tubes the reaction was read as negative.

Following this method a test was made of the blood of women committed to the New Jersey State Reformatory for Women and of other women reported to local health officials as reputed sources of venereal infection. The clinical diagnosis was made by physicians having a wide experience in the diagnosis of such cases. The results are as tabulated at top of page 301.

In addition to the recorded cases, this series included the examination of the blood of 12 cases of acute gonococcus infections (less than eight weeks duration):⁷ of these, 2 were doubtful, and 10

⁷ This test was performed upon the blood of a much smaller group of men under treatment at public clinics or by practicing physicians. The results correspond to those above, in the main:

Results of tests in men

TYPE OF CASE	NUMBER OF CASES	C-F REACTION		
		Posi- tive	Doubt- ful	Nega- tive
Acute (less than eight weeks).....	5	0	2	3
Chronic (more than eight weeks).....	6*	3*	3	0
Pronounced cured.....	3	1	0	2
Controls (negative).....	6	0	0	6

* One of these was a case of prostatitis of gonococcal origin of five years standing.

	NUMBER OF CASES	C-F REACTION		
		Posi- tive	Doubt- ful	Nega- tive
Group I. Clinically positive cases—sub-acute (of more than six weeks' duration, with profuse discharge).....	36	22	10	4
Group II. Other clinically positive cases—chronic (of more than six weeks' duration or approaching cure: with slight discharge).....	47	31	7	9
Group III. Recently cured cases (formerly clinically positive cases pronounced cured within eight weeks of test).....	5*	3	2	0
Group IV. Cured cases (formerly positive cases pronounced cured more than eight weeks previous to test).....	32	1†	0	31
Group V. Cases of other infections (this group includes cases of streptococcus or other bacterial infections in persons without history of recent gonorrhea).....	12	0	0	12
Group VI. Negative controls (persons with no evidence of any infection and no history of recent gonococcus infection).....	43	0	0	43

* Three of these cases later became negative and are included in group IV.

† Case had complete historectomy two years previously, showing no signs of persisting gonococcus infection.

negative. Of the latter ten, it was possible to reexamine the blood of six after the infection had persisted for more than eight weeks; when it was found that the blood had become positive or doubtful in all, and they are included in Group I.

In this series⁸ of 184 cases, 83 were clinically positive at the time the test was performed. Of these, only 15 per cent gave negative reactions; whereas, 63 per cent gave a definitely positive reaction; the remainder being doubtful. Our experience leads us to conclude that the test gives a higher percentage of positive results than any other laboratory procedure for the diagnosis of chronic gonorrhea.

There are, however, certain practical difficulties connected with the test itself, and with its interpretation, which lessen its value

⁸ Sera from 21 persons were found repeatedly anticomplementary, and are not included in this report.

as an aid to the control of the spread of gonorrhea. For instance, a negative result cannot rule out gonococcus infection: in at least 15 per cent of clinically positive cases clear-cut negatives were obtained. Again, a positive result does not prove active gonococcus infection: it can be interpreted only as indicating the presence of gonococcus antibodies in the blood, which means only that the person tested has *or has had* a gonococcus infection. Although the complement-fixation reaction is of distinct value to the clinician as an aid to diagnosis and prognosis, it is of questionable value as evidence upon which to base legal action. In this respect positive evidence when obtained either by the smear or culture is more definite and hence carries greater weight.

CONSIDERATION OF THE DIFFICULTIES ENCOUNTERED IN THE GONOCOCCUS COMPLEMENT-FIXATION TEST

The test is complicated by the failure of certain guinea pig sera to react. The reactions reported were made in conjunction with a routine Wassermann test in a State laboratory which uses the sera from a number of guinea pigs each week. On the day before the gonococcus complement-fixation test was made, a small amount of serum from each of five or six pigs was tested as described above. As a rule, only about half the sera proved satisfactory. This would mean that were the test not run in conjunction with a Wassermann test, these guinea pigs would have been wasted, hence the test can be run economically only when combined with a Wassermann test.

The degree of fixation is not marked. Apparently the strength of complement-fixation reaction between the gonococcus antigen and the typical gonorrheal patient's serum is not great; therefore, the reaction is obscured if too great an amount of complement is added. This means, in effect, that the complementary serum must be titrated very closely in order to make the test sensitive, yet not so closely as to create false positive findings resulting from the anticomplementary activity of both the antigen and the serum. In addition, sera transmitted in the mails in the usual manner occasionally have been found too anticomplementary to give a satisfactory reading, whereas the Wassermann test

could be performed satisfactorily upon them. We are of the opinion that this test cannot be conducted with any great degree of sensitivity or security by the partially trained laboratory technician, who can be entrusted under supervision to carry out the details of the routine blood Wassermann reaction.

The reading of the test is not easy. In only a small proportion of the tests read as positive was the fixation as complete as in the typical four plus Wassermann reaction. The degree of fixation not only varies in degree, difficult for any but an experienced operator to estimate, but the degree varies with the time of reading. If the reading is made soon after all the controls are hemolyzed, say fifteen minutes, reactions occasionally will be observed in which the degree of cloudiness indicates approximately 50 per cent inhibition of hemolysis, whereas after a half hour period, many will hemolyze to such an extent that they may be regarded as doubtful.

The difficulties encountered in carrying on the gonococcus complement-fixation test—false negative findings in 15 per cent of the cases; the question of interpretation when positive reactions are obtained; waste of guinea pigs; and difficulties in reading border-line reactions—prompted an attempt to determine the value of a variation of the complement-fixation test in which the antigen should consist of a saline suspension of the discharges from a suspected case of gonorrhea (the unknown quantity) and the serum instead of being unknown would be the immune rabbit serum.⁹ If the unknown antigen, in quantities less than one-quarter the anticomplementary dose, would fix complement in the presence of immune serum, the reaction could reasonably be called positive.

METHOD EMPLOYED IN CONDUCTING THE PUS-ANTIGEN COMPLEMENT-FIXATION TEST

For the purpose of this test, specimens of freshly expressed pus are collected upon sterile cotton swabs by saturating them with the discharge. When the swabs are received at the labora-

⁹ This modification of the complement-fixation test was suggested by Dr. John A. Kolmer.

tory they are dropped into tubes containing 3 cc. of sterile saline solution. When the pus is thoroughly emulsified, the swabs are removed and the suspensions are inactivated at 56°C. for one hour.¹⁰

Reagents and titration. The reagents used in this test are the same as those used in the blood complement-fixation reaction, and the titration procedure is the same. The hemolytic system consists of 0.1 cc. of a 5 per cent suspension of sheep erythrocytes and hemolytic amboceptor diluted so that two hemolytic units are contained in 0.1 cc. of the dilution. The activated cells are added in 0.2 cc. quantities.

In preparing the complement the guinea pig sera are tested for hemolytic activity, natural anti-sheep amboceptor, and fixability. The satisfactory sera are pooled and titrated; the dilution is made so that two units of complement are contained in 0.1 cc. of the dilution.

Description of the test. In the test as we conducted it, one rack of twelve tubes was used for each antigen; the front row of tubes containing varying quantities of antigen without immune serum to determine the anticomplementary unit. In each tube of the rear row is placed one-quarter the amount of antigen contained in the corresponding tube of the front row.

After a series of trials the following technique gave the most satisfactory reading. Tubes 1 to 12 are set up for each antigen; known positive (gonococcus antigen used in blood complement-fixation test); known negative (pus from streptococcus or staphylococcus infections); and unknown antigens. System controls are, of course, included in each test.

To read the test, the front row is first observed; as a rule tube 3, 4, or 5 is found to be the tube in which the anticomplementary action of the pus-antigen ceases.¹¹ For example, if tube 3

¹⁰ Specimens were inactivated in duplicate at 56°C. for one hour and at 80°C. for half an hour without an appreciable difference in the reaction. Therefore the lower temperature was selected because it was the more convenient to maintain.

¹¹ If all tubes in the front row show fixation of complement, it is necessary to dilute the antigen suspension and repeat the test. This occurred only occasionally in this series.

shows a degree of inhibition, tube 4 is selected as the unit to read if hemolysis is complete in it. The corresponding tube on the back row is then observed; if some degree of fixation is evident the test presumably is positive. The degree can be determined not only by the color and turbidity of this tube, but by the condition of the tubes on either side. For the purpose of this test, if 50 per cent of the cells remain unhemolyzed and the tube containing the next lower amount of antigen shows any degree of inhibition, the reaction is considered positive. If only a trace of fixation is evident, but the tube containing the next higher amount of antigen shows 50 per cent or more inhibition, the reaction is read as doubtful. If the tube is completely hemolyzed, the reaction is considered negative.

Pus-antigen complement-fixation test

	TUBE NUMBER	ANTIGEN	IMMUNE SERUM	COMPLE- MENT	SALINE	HEMOLY- TIC SYSTEM
		cc.	cc.	cc.		cc.
Anticomplemen- tary control (front row).....	1	0.4*	}	0.05*	Quantity to make all tubes to 0.4 cc.	0.1*
	2	0.4		0.1		0.2
	3	0.3				
	4	0.2				
	5	0.1				
	6	0.05				
Test (back row)...	7	0.2	}	0.1	Quantity to make all tubes to 0.4 cc.	0.2
	8	0.1				
	9	0.075				
	10	0.05				
	11	0.025				
	12	0.012				
System controls...			0.08	0.1	Quantity to make all tubes to 0.4 cc.	0.2
			0.04	0.1		
				0.1		

* To save materials, the complement and hemolytic system are halved instead of increasing the amount of antigen.

After adopting this method of conducting the reaction and reading the tests, it was tried upon specimens of pus from a series

of gonorrheal patients similar to those from whom specimens were obtained for the blood complement-fixation test. The results are tabulated:

	NUMBER OF CASES	C-F REACTION		
		Posi- tive	Doubt- ful	Nega- tive
Group A. Clinically positive cases—acute (of less than six weeks' duration; profuse purulent discharge; smear positive in majority of cases).....	10	6	3	1
Group I. Clinically positive cases—sub-acute (or more than six weeks' duration; profuse discharge)	13	6	5*	2
Group II. Clinically positive cases—chronic (of more than six weeks' duration, or approaching cure; with slight discharge).....	28	5	12	11†
Group III. Cured cases (formerly clinically positive cases pronounced cured).....	6	0	0	6
Group IV. Cases of other infections (this group contains cases with vaginal or cervical discharges not of gonococcal origin).....	4	0	0	4
Group V. Controls (purulent discharges produced by streptococcus or staphylococcus infections)..	5	0	0	5

* Including three from group A, strongly positive at the earlier test.

† Including the three from group A, and two from group I which were positive at the earlier test.

For convenience in comparing the blood and the pus-antigen complement-fixation tests the groups in the two series are given like numbers. The results recorded under Group A of the pus-antigen series indicate that the test is positive in a large proportion of the cases in which the pus contains great numbers of gonococci and as a result is rich in antigenic substance. Acute cases were not included as a group in the blood complement-fixation test, for negative or doubtful results were to be expected.

In group I over half the sub-acute cases gave negative or doubtful reactions in the pus-antigen test, whereas only 39 per cent gave similar reactions in the blood complement-fixation test.

In group II more than 80 per cent of the chronic cases gave negative or doubtful reactions in the pus antigen test, whereas only 34 per cent gave similar reactions in the blood complement-fixation test.

The rapid decrease in the complement-fixing power of the discharges from the few cases upon which the test could be repeated as the disease progressed is an indication that the antigenic property of the pus is dependent upon the presence of gonococci. The reactions obtained from the specimens included in groups III, IV, and V suggest that the test is specific for gonococcal infections, for false positive reactions did not occur.

COMMENT

A pus-antigen complement-fixation test would have definite advantages over a blood complement-fixation test if the former could be carried out in such a way as to give an equal proportion of positive findings in chronic cases. For this reason, the method and results of the present study are recorded in the hope that modifications of the test will occur to others, which may lead to a greater degree of sensitivity.

Positive reactions in the blood test can be interpreted only as indicating an existing or recently cured gonococcus infection. Similar findings from a test of the discharge from a suspected case could hardly be interpreted other than as evidence of an existing infection. Positive findings in this test are as significant as are those of microscopical or cultural tests; and in our experience are more readily obtained than in the latter procedures.

The pus-antigen reaction is simpler in technique than is the blood complement-fixation test. Positive and negative controls may be kept on hand by making stock antigens of gonococcus and streptococcus cultures. When the reaction is carried on in conjunction with the Wassermann test and the titrated reagents are at hand, the test requires but a short time to complete.

CONCLUSION

From the test of a limited series of cases, it may be assumed: (a) that the pus-antigen complement-fixation reaction does give positive results in cases of gonorrhea in which the pus is rich in gonococci and, presumably, in antigenic substances; (b) that the reaction is specific, in that discharges from other infections did

not give positive reactions; but (c) that in many chronic cases the discharges do not contain sufficient antigen to cause a reaction strong enough to be read with certainty. (d) The pus-antigen complement-fixation (as carried on by me) does not give as large a proportion of positive findings in sub-acute and chronic cases as does the blood complement-fixation reaction, and hence is inferior to the latter as an aid to the diagnosis of chronic gonorrhea.

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